CLAIMS

What is claimed is:

1. An isolated complex comprising heparin and the heparin binding proteins platelet factor 4 and thrombospondin-1.

2. The complex according to Claim 1 wherein the heparin binding proteins are isolated from mammalian blood.

3. The complex according to Claim 1 wherein the platelet factor 4 is an isolated protein selected from the group consisting of: a protein isolated from human platelets, a fragment prepared from a protein isolated from human platelets, a recombinant human protein, a synthetic peptide, a recombinant human variant protein and a chimeric human protein.

4. The complex according to Claim 1 wherein the thrombospondin-1 is an isolated protein selected from the group consisting of: a protein isolated from human platelets, a fragment prepared from a protein isolated from human platelets, a recombinant human protein, a synthetic peptide, a recombinant human variant protein and a chimeric human protein.

5. The isolated complex according to Claim 1 wherein the heparin platelet factor 4 and thrombospondin-1 are present at a ratio determined to be optimal for recognition by platelet factor 4/heparin/thrombospondin-1 ternary complex-reactive immunoglobulin present in a standardized positive control sample.

6. The isolated complex according to Claim 4 wherein the complex is preformed by combining 0.01-40 μg/ml platelet factor 4, 0.01-1.0 U/ml unfractionated heparin and 0.01-40 μg/ml thrombospondin-1.

7. The isolated complex according to Claim 5 wherein the complex is formed by combining 20 μg/ml human platelet factor 4, 0.03 U/ml unfractionated heparin and 1μg/ml human thrombospondin.

8. A method for diagnosing type-2 heparin-induced thrombocytopenia comprising detecting the presence of platelet factor 4/heparin/thrombospondin-1 ternary complex-reactive immunoglobulin in a plasma or serum sample obtained from a patient receiving a heparin drug, wherein the presence of said immunoglobulin is indicative of type 2 heparin induced thrombocytopenia.

9. The method of Claim 8 wherein the heparin drug is selected from the group consisting of porcine intestinal mucosal heparin, bovine lung heparin, metal heparinates, heparinoids, low molecular weight heparin, and heparin-like compounds.

10. The method of Claim 9 wherein said ternary complex-reactive immunoglobulin is of a human isotype selected from the group consisting of IgM, IgA, IgG and any combination thereof.

11. A method of detecting the presence of platelet factor 4/heparin/thrombospondin-1 ternary complex reactive immunoglobulin in a biological sample, comprising the steps of :

(a) contacting the biological sample with an antigen complex comprising platelet factor 4/heparin/thrombospondin-1 ternary complex, thereby producing a first combination;

(b) maintaining said first combination under conditions suitable to promote formation of antigen/antibody complexes referred to a first product;

(c) contacting the product of step (b) with detectably-labeled anti-human immunoglobulin reactive reagent specific for at least one isotype of human immunoglobulin, thereby producing a second combination;

(d) maintaining said second combination under conditions suitable to promote the binding of the detectably-labeled anti-human immunoglobulin reactive reagent to the antibody component of said first product; and

(e) detecting the presence of the detectably-labeled anti-human immunoglobulin reactive reagent, wherein detection of the reagent demonstrates the presence of ternary complex reactive immunoglobulin in the biological sample.

12. The method of Claim 11 wherein the platelet factor 4/heparin/thrombospondin-1 ternary complex comprises a heparin drug selected from the group consisting of porcine intestinal mucosal heparin, bovine lung heparin, a metal heparinate, a heparinoid, a low molecular weight heparin, a heparin-like compound and a combination of any of the preceding.

13. The method of Claim 11 wherein the platelet factor 4/heparin/thrombospondin-1 ternary complex comprises platelet factor 4 selected from the group consisting of a protein isolated from human platelets, a fragment prepared from a protein isolated from human platelets, a recombinant human protein, a synthetic peptide, a recombinant human variant protein, a chimeric human protein, and a combination of any of the preceding.

14. The method of Claim 11 wherein the thrombospondin-1 is an isolated protein selected from the group consisting of a protein isolated from human platelets, a fragment prepared from a protein isolated from human platelets, a recombinant

human protein, a synthetic peptide, a recombinant human variant protein and a chimeric human protein.

15. The method of Claim 11 wherein the antigen complex is preformed at a ratio determined to be optimal for recognition by platelet antigen complex-reactive immunoglobulin present in a standardized positive control sample.

16. The method of Claim 11 wherein the platelet factor 4/heparin/thrombospondin-1 ternary complex is immobilized on a solid support.

17. The method according to Claim 16 wherein the solid support is selected from the group consisting of: polycarbonate, polyallomer, polypropylene, polyvinyl, nylon, nitrocellulose, polystyrene and maleic anhydride activated polystyrene.

18. The method of Claim 11 wherein the ternary complex-reactive immunoglobulin has an isotype selected from the group consisting of: IgM, IgA, IgG and any combination thereof.

19. The method of Claim 11 wherein the detectably-labeled anti-human immunoglobulin reactive reagent specific for at least one isotype of human immunoglobulin comprises a reagent selected from the group consisting of: an anti-human immunoglobulin polyclonal antibody, an anti-human IgG polyclonal antibody, an anti-human IgM polyclonal antibody and an anti-human IgA polyclonal antibody.

20. The method of Claim 11 wherein the detectably-labeled anti-human immunoglobulin reactive reagent specific for at least one isotype of human immunoglobulin is selected from the group consisting of: intact immunoglobulin, $F(ab)_2$ fragments and F(ab) fragments.

21. The method of Claim 11 wherein the detectably-labeled anti-human immunoglobulin reactive reagent comprises a detectable label selected from the group consisting of: an enzyme, a radioactive molecule, an affinity ligand and a fluorophore.

22. The method according to Claim 21 wherein the enzyme is selected from the group consisting of: horseradish peroxidase, alkaline phosphatase, β-galactosidase, glucose oxidase.

23. The method of Claim 21 wherein the affinity ligand is biotin.

24. A method for the identification of an individual at risk for the occurrence of a thrombotic complication of type 2 heparin-induced thrombocytopenia, comprising the steps of:

(a) contacting a plasma or serum sample obtained from the individual with a platelet factor 4/heparin/thrombospondin-1 ternary complex, thereby producing a first combination;

(b) maintaining said first combination under conditions suitable to promote the formation of an antibody/antigen complex, wherein the antibody is derived from the plasma sample and the platelet factor 4/heparin/thrombospondin-1 ternary complex is the antigen; and

(c) detecting the antibody/antigen complex of step (b), wherein formation of the antibody/antigen complex is indicative of the individual being at risk for the occurrence of a thrombotic complication of type-2 heparin-induced thrombocytopenia.

25. The method of Claim 24, wherein detecting the presence of the antibody/antigen complex of step (b) is done by contacting the complex of (b) with a detectably

labeled immunologic reagent specific for binding to human IgG, IgA, IgM or any combination thereof.

26. The method of Claim 24 wherein the platelet factor 4/heparin/thrombospondin-1 ternary complex is immobilized on a solid phase.

27. The method according to Claim 24 wherein the solid phase is selected from the group consisting of: polycarbonate, polyallomer, polypropylene, polyvinyl, nylon, nitrocellulose, polystyrene and maleic anhydride activated polystyrene.

28. The method according to Claim 25 wherein said detectably labeled immunologic reagent is selected from the group consisting of: intact immunoglobulin, $F(ab)_2$ fragments and F(ab) fragments.

29. The method of Claim 24 wherein the platelet factor 4/heparin/thrombospondin-1 ternary complex comprises heparin selected from the group consisting of: porcine intestinal mucosal heparin, bovine lung heparin, a metal heparinate, a heparinoid, low molecular weight heparin, a heparin-like compound and a combination of any of the preceding.

30. The method of Claim 24 wherein the platelet factor 4/heparin/thrombospondin-1 ternary complex comprises platelet factor 4 selected from the group consisting of: a protein isolated from human platelets, a fragment prepared from a protein isolated from human platelets, a recombinant human protein, a synthetic peptide, a recombinant human variant protein, a chimeric human protein and a combination of any of the preceding .

31. The method of Claim 24 wherein the platelet factor 4/heparin/thrombospondin-1 ternary complex comprises thrombospondin-1 selected from the group consisting of: a protein isolated from human platelets, a fragment prepared from

a protein isolated from human platelets, a recombinant human protein, a synthetic peptide, a recombinant human variant protein, a chimeric human protein and a combination of any of the preceding.

32. The method of Claim 24 wherein the platelet factor 4/heparin/thrombospondin-1 ternary complex is preformed at a ratio determined to be optimal for the formation of a complex which is recognized by ternary complex-reactive immunoglobulin present in a standardized positive control sample.

33. The method of Claim 25 wherein the detectably labeled immunologic reagent comprises a label selected from the group consisting of: an enzyme, a radioactive molecule, an affinity ligand and a fluorophore.

34. A kit for an enzyme linked immunoadsorbent assay for detecting the presence of immunoglobulin reactive with a platelet factor 4/heparin/thrombospondin-1 antigen complex, comprising:
a buffered medium comprising heparin;
a buffered medium comprising isolated human PF4;
a buffered medium comprising isolated human TSP-1;
a wash medium formulated to reduce nonspecific binding;
at least one anti-human immunoglobulin reactive reagent detectably labeled with a reporter molecule, and having a specificity for at least one isotype of human immunoglobulin;
a standardized positive control comprising known amounts of ternary complex reactive antibody;
a negative control sample;
a substrate for the reporter molecule; and
a diluent reagent.

35. The kit according to Claim 34 further comprising a solid phase support suitable for the immobilization of a platelet factor 4/heparin/thrombospondin-1 ternary complex, wherein said solid phase support comprises a material selected from the group consisting of: polycarbonate, polyallomer, polypropylene, polyvinyl, nylon, nitrocellulose, polystyrene and maleic anhydride activated polystyrene.

36. A kit for detecting the presence of immunoglobulin reactive with a platelet factor 4/heparin/thrombospondin-1 antigen complex, comprising:
a solid phase support material on which a platelet factor 4/heparin/ thrombospondin-1 ternary complex has been immobilized; a buffered medium comprising isolated human TSP-1;
a wash medium formulated to reduce nonspecific binding;
at least one anti-human immunoglobulin reactive reagent detectably labeled with a reporter molecule, and having a specificity for at least one isotype of human immunoglobulin;
a standardized positive control comprising known amounts of ternary complex reactive antibody;
a negative control sample; and
a diluent reagent.